Nano structured Tin-Enabled Polytherapeutic Approach for Enhanced Treatment of Cutaneous Candidiasis

A Synopsis

on proposed plan of

Research work of

Doctor of philosophy

In

Pharmaceutical Sciences

Submitted by

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CERTIFICATE

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1. INTRODUCTION

1.1 Cutaneous Candidiasis

Every year, nearly 150 million individuals worldwide contract a fungal infection, resulting in approximately 1.7 million deaths [1]. More than 600 fungal species are known to infect humans, causing diseases that range from mild allergic reactions to severe, life-threatening conditions affecting the mucous membranes, skin, hair, and nails [2]. Among these, the genus *Candida* includes several pathogenic species responsible for candidiasis, a condition particularly prevalent in individuals with compromised immune systems [3].

Current antifungal treatments such as azoles, amphotericin B, allylamines, and echinocandins are effective against candidiasis; however, prolonged and repeated use has led to the emergence of drug-resistant *Candida* strains [4]. Notably, *Candida albicans* accounts for approximately 50% of *Candida* infections and, despite being treatable, demands increased attention due to its clinical significance [5].

A major global concern is the rapid development of multidrug resistance in pathogenic microorganisms through diverse mechanisms, including biofilm formation. These biofilms can actively expel therapeutic agents via efflux pumps, thereby reducing the drug concentration at the site of infection and compromising treatment efficacy.

Candidiasis is an opportunistic mycosis caused by *Candida* spp., affecting various anatomical sites such as the oral cavity, skin, reproductive tract, and gastrointestinal tract [6]. In severe cases, *Candida* infections may become systemic, resulting in life-threatening candidaemia. Globally, the annual incidence of *Candida* infections is estimated at approximately four million cases, with a mortality rate approaching 40%. This high fatality rate, combined with substantial drug costs and prolonged hospital stays, imposes a significant economic burden on healthcare systems [7].

Cutaneous candidiasis is a superficial fungal infection of the skin caused primarily by *Candida albicans*, though other *Candida* species may also be involved [8]. It typically occurs in warm, moist, and occluded areas of the body, such as skin folds (intertriginous regions), the groin, axillae, inframammary area, and interdigital spaces. The condition is more common in individuals with predisposing factors, including immunosuppression, diabetes mellitus, obesity, prolonged use of antibiotics or corticosteroids, and excessive sweating [9]. Clinically, cutaneous candidiasis

presents as erythematous, macerated patches with satellite pustules or papules at the periphery. The lesions are often pruritic, painful, and may be accompanied by fissuring or scaling. In severe or chronic cases, the infection can extend and recur frequently [10].

Among the etiological agents of candidiasis, *Candida albicans* remains the predominant pathogen due to its high prevalence and remarkable phenotypic plasticity [11]. Its colonization and infection potential are driven by a wide array of virulence factors, including thermotolerance (growth at 37 °C), morphological transition between yeast and hyphal forms, secretion of hydrolytic enzymes, haemolytic activity, tissue adhesion and invasion, evasion of host immune defenses, filamentation, and robust biofilm formation [12] **(Figure 1).**

Candida albicans is capable of forming biofilms under diverse environmental conditions on both biotic and abiotic surfaces, including host tissues, medical devices, dentures, and catheters [14,15]. These biofilms consist of structured communities of yeast, pseudo hyphae, and hyphae embedded within a self-produced extracellular polymeric matrix [16]. Biofilms not only protect fungal cells from the surrounding environment but also confer a high degree of resistance to conventional antifungal agents and components of the host immune system. This intrinsic resilience makes *C. albicans* biofilms a major contributor to recurrent candidiasis and a significant obstacle to effective antifungal therapy [13].

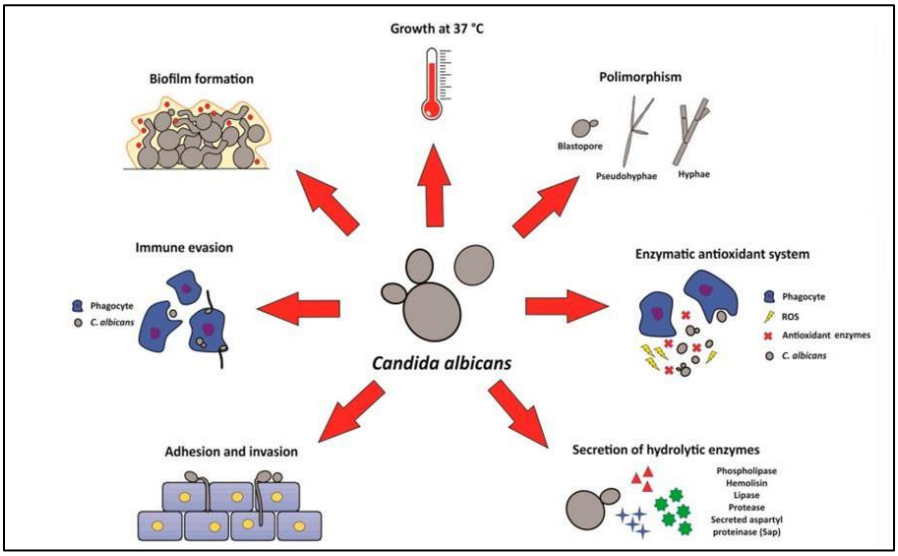


Figure 1. Major virulence factors of *C. albicans.*

1.2 Metal Nanoparticles

The development of nanodrugs and nanocarriers has opened new avenues in the biomedical field. Among these, metal nanoparticles have attracted considerable attention due to their potent activity against medically relevant fungal species, with applications extending to the pharmaceutical and agricultural sectors [14]. Nanoparticles are particulate dispersions or solid particles ranging from 10 to 100 nm in size, defined as objects with three external nanoscale dimensions. Metal nanoparticles possess unique physical and chemical properties, and their synthesis parameters can be tailored to modify characteristics such as size, shape, deformability, surface charge, hydrophobicity, and porosity. These factors, in turn, influence their stability, biocompatibility, drug-loading capacity, circulation time, biodistribution, cellular localization, and internalization.

Metal nanoparticles can be synthesized with a variety of core compositions, including noble metals (e.g., silver, gold), magnetic materials (e.g., iron), and metal oxides [15]. Their antifungal activity arises through multiple mechanisms: ion release, induction of oxidative and nitrosative stress, disruption of cell membranes and walls, inhibition of enzymatic activity, regulation of gene expression, depletion of ATP, and impairment of DNA, proteins, and mitochondrial function **(Figure 2)**.

Owing to these properties, metal nanoparticles have broad potential in the delivery of antimicrobial agents. Their combination with conventional antifungal drugs such as amphotericin B (AMB), caspofungin (CAS), and fluconazole (FCZ) has been shown to enhance efficacy against Candida albicans [16]. Furthermore, metal nanoparticles demonstrate significant potential in overcoming antifungal resistance and in eradicating C. albicans biofilms formed on mucosal surfaces and medical devices [17]. Recent research has particularly focused on silver (Ag), gold (Au), and iron (Fe) nanoparticles for the management of *C. albicans* infections.

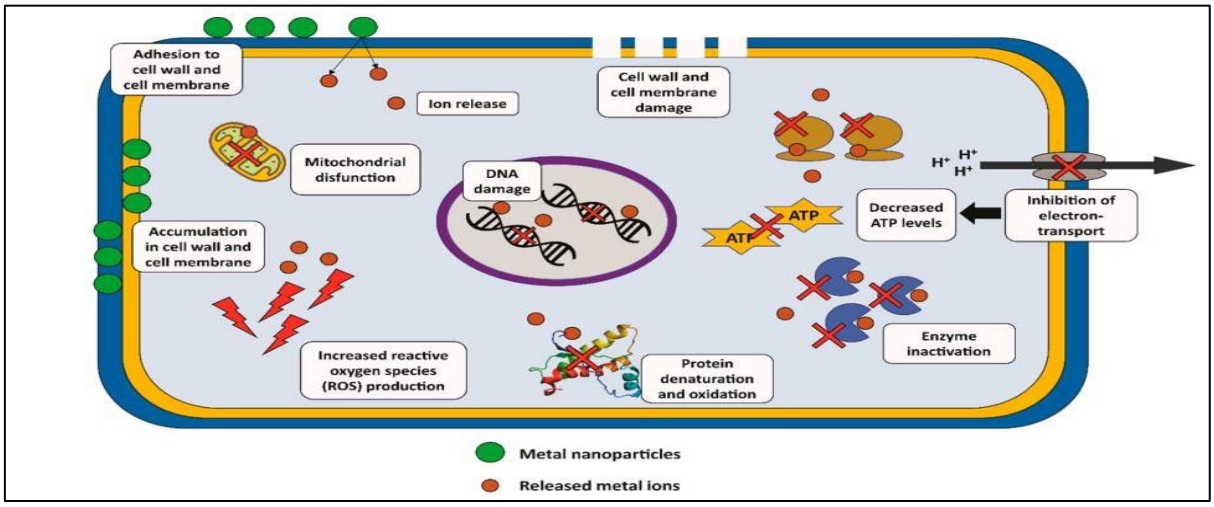


Figure 2. Main mechanisms of action of metallic nanoparticles against *C. albicans.*

1.2.1 Tin nanoparticles

Tin (Sn) is a post-transition metal with unique electrical, chemical, and structural properties. In recent years, tin nanoparticles (SnNPs) have gained interest due to their size-dependent properties that differ significantly from bulk tin. Tin oxide nanoparticles, often known as SnO2, have gained significant attention as a versatile and promising nanomaterial due to their extensive applicability across various fields. In recent years, there has been a notable increase in research efforts aimed at investigating the synthesis, characteristics, and uses of SnO2 nanoparticles [18].

1.2.1.1 Properties of Tin Nanoparticles

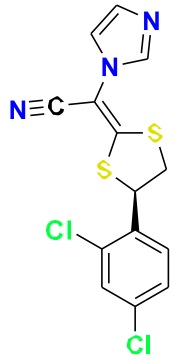
- Size-dependent optical and electronic behavior due to quantum confinement.
- High surface-to-volume ratio enhances catalytic activity.
- Oxidation tendency: SnNPs can easily oxidize to SnO or SnO_2, which can be both a challenge and an opportunity for applications.
- Good electrical conductivity, making them promising in energy storage devices.
- Antimicrobial potential against bacteria and fungi due to ROS generation and cell wall disruption.

1.3 Drug Profile: Luliconazole

Luliconazole is a novel imidazole antifungal agent belonging to the azole class of drugs (**Figure 1.3)** [19]. It was first developed in Japan and has gained clinical significance due to its broad

spectrum activity against dermatophytes, Candida species, and certain molds. Structurally, Luliconazole is an imidazole derivative that inhibits fungal lanosterol 14α-demethylase, a key enzyme involved in the biosynthesis of ergosterol, which is an essential component of the fungal cell membrane [20]. By disrupting ergosterol synthesis, Luliconazole compromises membrane integrity, leading to fungal cell death. Compared to conventional azole antifungals, Luliconazole demonstrates high potency at lower concentrations, rapid fungicidal activity, and excellent skin penetration. It is primarily used topically in the treatment of superficial fungal infections, including tinea pedis (athlete's foot), tinea cruris (jock itch), and tinea corporis (ringworm) [21]. The drug is well tolerated with minimal systemic absorption, making it a safe option for long-term topical therapy.

The clinical importance of Luliconazole lies in its ability to overcome certain limitations of earlier antifungals, such as poor patient compliance due to longer treatment durations and resistance development. Its once-daily dosing regimen and shorter treatment period offer significant advantages in improving adherence and therapeutic outcomes.



(*R*,*E*)-2-(4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene)-2-(1*H*-imidazol-1-yl)acetonitrile
luliconazole

Figure 1.3: Chemical Structure of luliconazole.

Drug Name	Luliconazole
Chemical Class	Azole antifungal (Imidazole derivative)
IUPAC Name	(R,E)-2-(4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-(1H-imidazol-1-yl) acetonitrile

Molecular Formula	$C_{14}H_9Cl_2N_3S_2$
Molecular Weight	354.3 g/mol
Appearance	White to pale yellow crystalline powder
Mechanism of Action	Inhibits lanosterol 14-α-demethylase (a cytochrome P450 enzyme), blocking ergosterol synthesis → disrupts fungal cell membrane integrity.
Antifungal Spectrum	Broad spectrum: Dermatophytes (Trichophyton, Microsporum, Epidermophyton), Candida spp., Aspergillus spp.
Indications	Topical treatment of: • Tinea pedis (athlete's foot) • Tinea cruris (jock itch) • Tinea corporis (ringworm) • Onychomycosis (in some formulations under investigation)
Dosage Form	1% topical cream, lotion, solution, gel (for nails/skin)
Usual Adult Dose	Apply thin layer once daily to affected area for 1–2 weeks (depending on condition).
Pharmacokinetics	• Absorption: Minimal systemic absorption when applied topically • t½: Not well-defined due to low systemic exposure • Metabolism: Limited, via hepatic pathways if absorbed • Excretion: Feces and urine (minor, unmetabolized)
Advantages	• Once-daily dosing • Short treatment duration (1–2 weeks) • High potency against dermatophytes • Minimal systemic absorption (safe for topical use)
Adverse Effects	Local irritation, itching, burning, contact dermatitis, erythema (rare systemic effects)
Contraindications	Hypersensitivity to luliconazole or other imidazoles
Pregnancy Category	Category C (use only if benefits outweigh risks)
Regulatory Status	FDA-approved (2013) for topical use in dermatophytosis

1.4 Eugenol

Eugenol is a naturally occurring phenolic compound mainly found in clove oil, nutmeg, cinnamon, and basil [22]. It possesses a characteristic spicy aroma and is widely used as a flavoring agent in food and beverages. Due to its antiseptic and analgesic properties, eugenol has long been used in dentistry, particularly in dental cements and mouthwashes. Pharmacologically, it exhibits antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, making it a promising bioactive compound. Its ability to inhibit fungal growth has also gained attention for potential applications in managing resistant infections. Eugenol is lipophilic in nature, which aids in membrane penetration but also limits its solubility in water. At higher concentrations, it can cause irritation or cytotoxic effects, which necessitates controlled use. Overall, eugenol is a versatile natural molecule with significant therapeutic and industrial importance. **Figure 1.4** represent the chemical structure of Chemical Structure of Eugenol.

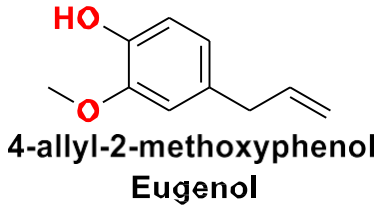


Figure 1.4: Chemical Structure of Eugenol.

Drug Name	Eugenol
IUPAC Name	4-allyl-2-methoxyphenol
Molecular Formula	$C_{10}H_{12}O_2$
Molecular Weight	164.20 g/mol
Source	Major constituent of clove oil; also found in nutmeg, cinnamon, basil, and bay leaf
Physical Appearance	Colorless to pale yellow oily liquid with spicy clove-like odor
Solubility	Slightly soluble in water; soluble in ethanol, ether, chloroform, and oils

Log P	~2.27
Melting Point	−7.5 °C
Boiling Point	~254 °C
Mechanism of Action	Acts as an antioxidant, anti-inflammatory, antimicrobial, analgesic; inhibits prostaglandin synthesis and disrupts microbial cell membranes
Pharmacological Activities	Antioxidant, Antimicrobial (antifungal, antibacterial), Analgesic, Anti-inflammatory, Anticancer, Local anesthetic
Therapeutic Uses	Dental analgesic, antiseptic, treatment of toothache, sore gums, mouth ulcers; potential anticancer and antifungal agent
Toxicity	High doses may cause cytotoxicity, hepatotoxicity, and contact Dermatitis
Dosage Forms	Oils, topical gels, mouthwashes, dental cements, experimental Nanoformulations
Regulatory Status	FDA-approved as a flavoring agent; used in pharmaceuticals, cosmetics, and food industry (GRAS status)

1.5 Diclofenac

Diclofenac is a widely used nonsteroidal anti-inflammatory drug (NSAID) belonging to the phenylacetic acid class. It is commonly prescribed for the management of pain and inflammation associated with musculoskeletal disorders, arthritis, postoperative pain, and dysmenorrhea. Diclofenac exerts its pharmacological action primarily through reversible inhibition of cyclooxygenase enzymes (COX-1 and COX-2), thereby reducing the synthesis of prostaglandins that mediate inflammation, pain, and fever. Due to its efficacy, multiple dosage forms such as oral tablets, injections, topical gels, suppositories, and ophthalmic drops are available. However, long-term or high-dose use is associated with gastrointestinal, hepatic, renal, and cardiovascular adverse effects, making careful monitoring and rational use important in clinical practice [23].

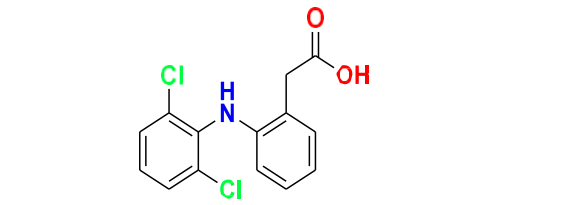


Figure 1.5: Chemical Structure of Diclofenac.

Drug name	Diclofenac
Category / Class	Nonsteroidal Anti-inflammatory Drug , Analgesic, Antipyretic
IUPAC Name	2-(2-((2,6-dichlorophenyl)amino) phenyl) acetic acid
Molecular Formula	$C_{14}H_{11}Cl_2NO_2$
Molecular Weight	296.15 g/mol
Mechanism of Action	Inhibits cyclooxygenase (COX-1 and COX-2), thereby decreasing prostaglandin synthesis → reduces inflammation, pain, and fever.
Therapeutic Uses	Relief of pain and inflammation in osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, dysmenorrhea, postoperative pain, acute musculoskeletal disorders.
Pharmacokinetics	Absorption: Well, absorbed orally Bioavailability: ~50–60% (first-pass metabolism) Protein binding: >99% Half-life: 1–2 hrs Metabolism: Hepatic (CYP2C9) Excretion: Mainly urine, some via bile/feces
Routes of Administration	Oral (tablets, capsules), Parenteral (injections), Topical (gels, patches), Ophthalmic drops, Suppositories
Adverse Effects	Gastrointestinal irritation, ulcers, bleeding, hepatotoxicity, nephrotoxicity, cardiovascular risk (MI, stroke), skin reactions

Contraindications	Hypersensitivity to diclofenac, history of GI bleeding or ulcers, severe hepatic/renal impairment, heart failure, pregnancy (3rd trimester)
Drug Interactions	Increased risk of bleeding with anticoagulants; reduced efficacy of antihypertensives/diuretics; increased nephrotoxicity with cyclosporine/methotrexate.
Regulatory Status	Widely approved; available in multiple formulations globally (both prescription and OTC depending on strength and formulation).

2.1 Literature review

Manjushree *et al.*, (2025) developed ketoconazole-loaded menthosomes as a novel ultra-deformable vesicular system to enhance transdermal drug delivery. A 2^3 full factorial design optimized formulation parameters, yielding vesicles of 107.6 nm with good stability and high entrapment efficiency (76.9%). Ex vivo and in vitro studies confirmed improved skin permeation, drug release, and antifungal activity against *Candida albicans*. Overall, menthosomes show strong potential as effective carriers for enhancing topical antifungal therapy (Manjushree et al. 2025).[24]

Jahrodi *et al.*, (2024) investigated eugenol-encapsulated chitosan nanoparticles (EECNPs) were developed to enhance the antifungal activity of eugenol against *A. fumigatus*. The nanoparticles showed optimal properties at a 1:1 eugenol/chitosan ratio with high encapsulation efficiency (43.1%) and loading capacity (30.8%), having an average size of ~300 nm and stable zeta potential. Antifungal assays revealed significant inhibition of fungal growth at MIC values of 150–300 µg/mL, with complete growth suppression observed after 12 h in time-kill studies. Mechanistic studies confirmed that EECNPs caused oxidative stress, potassium leakage, and membrane/cell wall damage, contributing to strong antifungal action. Overall, the formulation improved eugenol's half-life and safety profile, highlighting its potential as a natural antimicrobial agent against *A. fumigatus* [25].

Manjit *et al.*, (2024) researched nanofiber-based anti-infective dressing was developed using polyvinyl alcohol/chitosan hydrogel co-loaded with silver nanoparticles and luliconazole-PLGA nanoparticles for Candida-infected diabetic foot ulcers. The nanofibers exhibited favorable pH, hydrophilicity, and strong adhesion to wound sites, while demonstrating potent antifungal and antibiofilm effects against multiple Candida strains. They showed excellent water retention, vapor transmission, and sufficient payload capacity with controlled release confirmed by in-vivo imaging. Biocompatibility and enhanced keratinocyte proliferation were validated through in-vitro studies. In-vivo evaluation further revealed accelerated wound healing by combining antifungal activity with improved blood flow and cellular proliferation [26].

Mingshan Li et al, (2024) Diclofenac, a nonsteroidal anti-inflammatory drug, is commonly prescribed for managing osteoarthritis, rheumatoid arthritis, and post-surgical pain. However, oral administration of diclofenac often leads to adverse effects. This study introduces an innovative nano-in-micro approach to create diclofenac nanoparticle-loaded microneedle patches aimed at localized, sustained pain relief, circumventing the drawbacks of oral delivery. The nanoparticles were produced *via* wet-milling, achieving an average size of 200 nm, and then incorporated into microneedle patches. These patches showed improved skin penetration in *ex vivo* tests using Franz-cell setups compared to traditional diclofenac formulations. *In vivo* tests on rats revealed that the nanoparticle-loaded microneedle patches allowed for quick drug uptake and prolonged release, maintaining drug levels in tissues for up to 72 h. With a systemic bioavailability of 57 %, these patches prove to be an effective means of transdermal drug delivery. This study highlights the potential of this novel microneedle delivery system in enhancing the treatment of chronic pain with reduced systemic side effects [27].

Gerard Esteruelas *et al*, (2023) Cancer is identified as one of the main causes of death worldwide, and an effective treatment that can reduce/eliminate serious adverse effects is still an unmet medical need. Diclofenac, a non-steroidal anti-inflammatory drug , has demonstrated promising antitumoral properties. However, the prolonged use of this NSAID poses several adverse effects. These can be overcome by the use of suitable delivery systems that are able to provide a controlled delivery of the payload. In this study, Diclofenac was incorporated into biodegradable polymeric nanoparticles based on PLGA and the formulation was optimized using a factorial design approach. A monodisperse nanoparticle population was obtained with a mean size of ca. 150 nm

and negative surface charge. The release profile of diclofenac from the optimal formulation followed a prolonged release kinetics. Diclofenac nanoparticles demonstrated antitumoral and antiangiogenic properties without causing cytotoxicity to non-tumoral cells, and can be pointed out as a safe, promising and innovative nanoparticle-based formulation with potential antitumoral effects [28].

Mahmood *et al.*, (2021) formulated to overcome the limitations of conventional dosage forms, LUL-loaded lyotropic liquid crystalline nanoparticles (LCNP) were developed and optimized using Central Composite Design. The nanoparticles exhibited a particle size of 181 ± 12.3 nm with high entrapment efficiency (91.49 ± 1.61 %) and sustained drug release up to 54 h. Ex-vivo studies showed significantly enhanced skin permeation, with flux values approximately two-fold higher than marketed cream. Moreover, drug retention in the stratum corneum, epidermis, and deeper skin layers was markedly greater, with AUC values up to 6.5-fold higher in the dermis. Overall, the findings confirm LCNP as a promising carrier for improved antifungal delivery via the transdermal route [29].

Sharma *et al.*, (2021) developed luliconazole-loaded solid lipid nanoparticles were developed using a microwave-assisted technique with stearic acid and Pluronic F-68. The investigation revealed that microwave power significantly influenced particle size and zeta potential, while Pluronic F-68 concentration had a lesser effect, and neither factor affected drug entrapment. The optimized nanoparticles showed a particle size of 91.39 nm, zeta potential of −20.1 mV, and high entrapment efficiency of 96.68%. They achieved complete drug release within 24 h at pH 7.4 following Higuchi's diffusion-controlled kinetics, and TEM confirmed their spherical morphology. The formulation demonstrated potent antifungal activity, with MIC values of 6.25 µg/mL against *Candida albicans* and 12.5 µg/mL against *Aspergillus niger* [30].

Kumar (2020) aimed to overcome the bioavailability limitations of luliconazole by developing a topical solid lipid nanoparticles (SLN) gel formulation. Luliconazole-loaded SLNs were prepared using stearic acid and poloxamer 188 and optimized for high entrapment efficiency. The optimized formulation (F6) exhibited 92.13% entrapment, particle size ~344.3 nm, PDI 0.168, and zeta potential ~18.8 mV. The G3 gel containing 1.5% carbopol 934 showed 91.39% entrapment efficiency and sustained drug release of ~79.57% over 24 hours. Anti-fungal evaluation confirmed

strong inhibitory activity against *Candida albicans,* establishing G3 gel as a promising topical treatment [31].

Kumar *et al.*, (2019) formulated luliconazole nanocrystal (LNC) incorporated hydrogel was developed to enhance topical antifungal therapy. The study highlighted that LNC-based gel exhibited improved solubility, dissolution, skin retention, and antifungal activity compared to raw drug and coarse formulations. The prepared gel was homogeneous, safe, non-irritant, and achieved about 88% drug entrapment. Notably, LNC hydrogel showed higher skin drug retention (62.17%) and superior zone of inhibition (44.25 mm) compared to conventional gels. Overall, LNC-loaded hydrogel proved to be a promising drug delivery system for effective management of fungal infections [32].

Khanna and Bharti (2014) studied luliconazole is an imidazole antifungal agent with a unique ketene dithioacetate structure, offering higher potency as the R-enantiomer compared to lanoconazole. Preclinical and clinical studies show excellent activity against dermatophytes, Candida, Malassezia, and Aspergillus, with fungicidal efficacy against *Trichophyton* spp. comparable to terbinafine. Clinical trials confirm its superiority over placebo and comparable or better efficacy than terbinafine, with effective short-term treatment using 1% cream once daily. It demonstrates good local tolerability, minimal side effects, and no systemic toxicity. Approved in Japan in 2005 and later by the US FDA, luliconazole is now widely used for dermatophytosis, including tinea pedis, cruris, and corporis [33].

Garg *et al.*, (2014) formulated EG–SLNs were incorporated into carbopol hydrogels, and their physicochemical properties were characterized using dynamic light scattering, TEM, and AFM. The rheological and mechanical studies confirmed that the EG–SLN hydrogels followed pseudoplastic flow behavior as per the Carreau model. Epidermal targeting studies revealed significantly higher accumulation of eugenol in the epidermis with EG–SLN-enriched hydrogels compared to eugenol–cyclodextrin complex and almond oil solution. Occlusion studies further demonstrated improved skin hydration in EG–SLN hydrogel-treated samples. Thus, EG–SLN hydrogels show strong potential as epidermal-targeted formulations for effective management of skin fungal infections [34].

Gag *et al.*, (2011) developed eugenol-loaded solid lipid nanoparticles (SLNs) were developed and characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, drug release, and antifungal activity. The incorporation of liquid lipid (caprylic triglyceride) with solid lipid (stearic acid) reduced crystallinity as confirmed by FT-IR, DSC, and XRD. SLN2 (binary lipid matrix) exhibited smaller particle size, higher encapsulation efficiency, and faster drug release compared to SLN1 (single lipid matrix). TEM analysis confirmed spherical morphology, while in vivo studies using an oral candidiasis model showed superior antifungal efficacy of SLNs compared to eugenol solution. Overall, the results demonstrate that lipid-based nanocarriers enhance eugenol's antifungal potential effectively [35].

3.1 Background and Rationale

Cutaneous candidiasis, mainly caused by *Candida albicans*, is a common superficial fungal infection. Current antifungal therapies (azoles, amphotericin B, allylamines, echinocandins) face issues such as drug resistance, poor tissue penetration, relapse, and toxicity.

- **Luliconazole**: a potent imidazole antifungal that disrupts ergosterol synthesis, but suffers from poor solubility and limited skin retention.
- **Eugenol**: a natural phenolic compound with antifungal, antioxidant, and anti-inflammatory activity; it disrupts membranes and reduces biofilm formation but has low stability and rapid metabolism.
- **Diclofenac**: Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID). This medicine works by reducing substances in the body that cause pain and inflammation.

Tin nanoparticles (SnNPs) add value by providing:

- Inherent antifungal and antimicrobial activity (ROS generation, membrane disruption),
- Biocompatibility,
- High surface area for drug loading,
- Potential to enhance drug penetration into infected tissue.

Rationale: A single nanoplatform combining luliconazole and eugenol with SnNPs offers multifunctional therapy:

- Luliconazole: inhibits ergosterol biosynthesis,
- Eugenol : enhances uptake, reduces resistance, and adds anti-inflammatory action,

- Diclofenac: Enhances Antiproliferative Activity and anti-inflammatory action.
- SnNPs : act as carrier and co-therapeutic agent (antifungal, penetration enhancer).

This synergistic approach addresses limitations of monotherapy, improves solubility and retention, enables sustained release, lowers dosing frequency, and minimizes resistance.

3.2 Aim & Objectives

3.2.1 Aim

Nanostructured Tin-Enabled Polytherapeutic Approach for Enhanced Treatment of Cutaneous Candidiasis.

3.2.2 Objectives

1. To perform preformulation studies and Evaluate drug–excipient compatibility.
2. To synthesize and optimize Luliconazole–Eugenol loaded Tin Nanoparticles.
3. To synthesize and optimize Diclofenac Loaded Tin Nanoparticles
4. To characterize nanoparticles for: particle size, PDI, zeta potential, % entrapment efficiency, morphology (TEM), UV spectrum, and drug release.
5. To develop and characterize a topical gel containing the nanoparticles.
6. To study the *in vitro* drug release profile and stability of the nanoparticle gel formulation.
7. To check antifungal activity against *Candida albicans* by Alamar Blue Assay.

3.3 Plan of work

3.3.1 Pre-formulation studies

- Organoleptic properties
- Melting point determination
- Fourier transformation infrared spectroscopy (FTIR)
- Solubility study
- Finding the absorption maxima (π max)

3.3.2 Preparation of Luliconazole and Eugenol-loaded Tin Nanoparticles

3.3.3 Preparation of Diclofenac loaded Tin Nanoparticles

3.3.4 To optimize the Luliconazole based Tin Nanoparticles by response surface methodology

3.3.5 Characterization of Diclofenac, Luliconazole and Eugenol -loaded Tin Nanoparticles

- UV-Absorption spectrum of SnO_2
- Particle size distribution, PDI
- Zeta potential study
- % Entrapment efficiency
- Transmission electron microscopy (TEM)
- Drug release study

3.3.6 Formulate and characterize a topical gel containing Diclofenac, Luliconazole and Eugenol Based Tin Nanoparticles

- Organoleptic Properties
- pH
- Spreadability
- Viscosity
- Gel strength
- Drug Content
- In-vitro drug release study

4. Material and Methods

Luliconazole, Eugenol and Diclofenac will be purchased from the Yellow Chem. India. Polyvinylpyrrolidone K30, $NaBH_4$, PVP/Poloxamer, water, ethanol, Carbopol 934, and triethanolamine will obtain from central drug house Pvt. Ltd, India.

4.1 Method of preparation of Luliconazole and Eugenol-loaded tin nanoparticles

A 100 mL lab-scale batch of Luliconazole and Eugenol-loaded tin nanoparticles (Lu-Eg-SnNPs) will be prepared by chemical reduction of stannous chloride in a sterically stabilized medium followed by drug co-loading. Briefly, stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$, 0.225 g; 1.0 mmol) will dissolved in a deoxygenated hydroalcoholic phase (60 mL purified water + 20 mL absolute ethanol) containing polyvinylpyrrolidone K30 (PVP, 0.80 g; 1.0% w/v) and Poloxamer-188 (0.20 g; 0.25% w/v) under nitrogen at 0–5 °C. Separately, eugenol (0.10 mL; ~106 mg) will dissolved in ethanol (5 mL) and added to the tin precursor solution as a hydrophobic ligand/co-stabilizer while maintaining gentle stirring (800 rpm) for 10 min. Fresh sodium borohydride solution ($NaBH_4$, 0.152 g; 4.0 mmol in 10 mL ice-cold water) will then introduced dropwise over 15 min under nitrogen; the mixture darkened to gray-black, indicating SnNP formation. After 30 min of maturation at 0–5 °C, a luliconazole stock (50 mg in 10 mL ethanol with 0.1% v/v triethylamine to aid solubilization) will added slowly and the dispersion was stirred for 2 h at room temperature, protected from light, to enable co-loading/adsorption within the PVP/Poloxamer corona and at the eugenol-rich shell. The crude dispersion will be dialyzed (MWCO 12–14 kDa) against 1 L of water:ethanol (90:10) for 2 h with two medium changes to remove residual borates, unbound drug, and solvents, then concentrated by centrifugation (15,000 g, 30 min, 4 °C) and re-dispersed in phosphate buffer (pH 6.5) to a final volume of 50 mL. Theoretical composition at this scale corresponds to ~1.6 mg/mL Sn precursor, 16 mg/mL PVP, 4 mg/mL Poloxamer, 2.1 mg/mL eugenol, and 1.0 mg/mL luliconazole (targeting ~5 % w/w Lu and ~10 % w/w Eg relative to total solids prior to purification). Dispersions will be stored at 4 °C under nitrogen until further characterization.

4.2 Preparation of Diclofenac loaded Tin Nanoparticles

A 100 mL lab-scale batch of Diclofenac-loaded tin nanoparticles will be prepared by chemical reduction of stannous chloride in a sterically stabilized medium followed by drug co-loading. Briefly, stannous chloride dihydrate ($SnCl_2 \cdot 2H_2O$, 0.225 g; 1.0 mmol) will dissolved in a deoxygenated hydroalcoholic phase (60 mL purified water + 20 mL absolute ethanol) containing polyvinylpyrrolidone K30 (PVP, 0.80 g; 1.0% w/v) and Poloxamer-188 (0.20 g; 0.25% w/v) under nitrogen at 0–5 °C. Diclofenac (0.10 mL; ~106 mg) will dissolved in ethanol (5 mL) and added to the tin precursor solution as a hydrophobic ligand/co-stabilizer while maintaining gentle stirring (800 rpm) for 10 min. Fresh sodium borohydride solution ($NaBH_4$, 0.152 g; 4.0 mmol in 10 mL ice-cold water) will then introduced dropwise over 15 min under nitrogen; the mixture darkened to gray-black, indicating SnNPs formation. The crude dispersion will be dialyzed (MWCO 12–14 kDa) against 1 L of water: ethanol (90:10) for 2 h with two medium changes to remove residual borates, unbound drug, and solvents, then concentrated by centrifugation (15,000 g, 30 min, 4 °C) and re-dispersed in phosphate buffer (pH 6.5) to a final volume of 50 mL. Theoretical composition at this scale corresponds to ~1.6 mg/mL Sn precursor, 16 mg/mL PVP, 4 mg/mL Poloxamer, 2.1 mg/mL Diclofenac ~10 % w/w Dic relative to total solids prior to purification). Dispersions will be stored at 4 °C under nitrogen until further characterization.

4.3 Preparation of tin nanoparticles loaded Gel

Carbopol-940 was used for the preparation of luliconazole tin nanoparticles loaded hydrogel. The method involves mixing Carbopol-940 (1−2%w/v) in 10 ml of tin nanoparticles dispersion for 10min at1000rpm on a mechanical stirrer. The pH of the hydrogel was adjusted to 5.5 using triethanolamine

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